Effect of Ductus Ligation on Cardiopulmonary Function in Premature Baboons

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Rationale: The role of the patent ductus arteriosus in the development of chronic lung disease in surfactant-treated premature newborns remains unclear.

Objective: To examine the effects of ductus ligation on cardiopulmonary function and lung histopathology in premature primates.

Methods: Baboons were delivered at 125 d, (term = 185 d) treated with surfactant, and ventilated for 14 d. Serial echocardiograms and pulmonary function tests were performed. Animals were randomized to ligation ($n = 12$) or no ligation (controls, $n = 13$) on Day 6 of life. Necropsy was performed on Day 14.

Results: Compared with nonligated control animals, ligated animals had lower pulmonary-to-systemic flow ratios, higher systemic blood pressures, and improved indices of right and left ventricular performance. The ligated animals tended to have better compliance and ventilation indices for the last 3 d of the study. There were no differences between the groups in proinflammatory tracheal cytokines (interleukin [IL] 6 and IL-8), static lung compliance, or lung histology. **Conclusion:** Although a persistent patent ductus arteriosus results in diminished cardiac function and increased ventilatory requirements at the end of the second week of life, ligation on Day 6 had no measurable effect on the histologic evolution of chronic lung injury in this 14-d baboon model.

Keywords: bronchopulmonary dysplasia; chronic lung disease; patent ductus arteriosus; premature newborn

Although a patent ductus arteriosus (PDA) increases fluid and protein efflux from the pulmonary vasculature in preterm infants (1, 2), a compensatory increase in pulmonary lymph flow appears to prevent pulmonary edema during the first 72 h after delivery in both premature animals (3, 4) and humans (5). However, a symptomatic left-to-right ductus shunt that persists beyond the first week of life increases the likelihood of edema formation and respiratory compromise. Several studies (performed in the presurfactant era) found that, by 7 d after birth, a PDA can alter pulmonary mechanics (6–8). In addition, preterm infants with a PDA have increased ventilatory needs compared with infants whose PDA has been closed (6, 7, 9–12). Whether these alterations are due to increasing pulmonary edema or to more permanent histopathologic changes is currently unknown.

Chronic pulmonary histopathologic changes consisting of alveolar simplification, fibrosis, and chronic inflammation frequently follow preterm birth (13). These changes, known as bronchopulmonary dysplasia (BPD), appear to be due to inflammatory and repair processes that interfere with normal lung development (14–17). Airway aspirates from infants that develop BPD contain proinflammatory cytokines (e.g., interleukin [IL] 6 and IL-8) during the first days after delivery (18), and ventilation of preterm animals initiates a similar inflammatory response (13, 18). Although numerous studies have found an association between the presence of a PDA and development of BPD, there is little information suggesting a cause-and-effect relationship (19, 20). Infants with a persistent PDA frequently require prolonged ventilatory support similar to infants with a more severe form of BPD; however, previous studies have failed to demonstrate consistent worsening in the radiographic features of BPD among infants with a persistent PDA (11, 12, 21). Studies to unravel the mechanisms responsible for the late pulmonary deterioration associated with a persistent PDA have been hampered by both the absence of randomized controlled clinical trials that address this question and by the absence of an appropriate animal model.

The premature baboon (delivered at 125 d gestation; term = 185 d) has a similar postnatal course as humans delivered between 25 and 27 wk of gestation. As in very-low-birthweight human infants, the ductus arteriosus of the premature baboon has only a 20 to 30% chance of closing spontaneously after birth (22). Despite surfactant treatment, low tidal volume breathing, and low supplemental oxygen administration, premature baboons develop pulmonary histopathologic changes during the first 2 wk after delivery that are similar to those described in premature human infants (13, 23). The purpose of the present study was to examine the effectiveness of surgical ligation as a means of treating infants with a PDA by assessing its effects on cardiopulmonary function and the development of lung injury in mechanically ventilated premature baboons during the first 2 wk after delivery. We hypothesized that preterm baboons would have improved cardiopulmonary function after ductus ligation compared with unligated animals.

Some of the results of these studies have been previously reported in the form of an abstract (24).

METHODS

A complete description of the methods is provided on the online supplement. All studies were performed at the Southwest Foundation for Biomedical Research Primate Center in San Antonio, Texas. Details of animal care have been published elsewhere (13, 23). Briefly, timed baboon (*Papio papio*) pregnancies were delivered at 125 ± 2 d and mechanically ventilated for 14 d. At birth, they were weighed, sedated, intubated, and given surfactant (Survanta; courtesy of Ross Laboratories, Columbus, OH) before initiation of ventilator support (InfantStar; Infrasonics, San Diego, CA). Ventilator adjustments were made on the basis of chest radiograph, clinical examination, arterial blood gas measurement, and tidal volume measurement. We chose to study the animals

(Received in original form February 14, 2005; accepted in final form September 20, 2005)

Supported in part by National Institutes of Health grants HL 63399, HL56061, and HL52636, the BPD Resource Center, and grant P51RR13986 from the Primate Center facility.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 172, pp 1569–1574, 2005

Originally Published in Press as DOI: 10.1164/rccm.200502-2300C on September 22, 2005
Internet address: www.atsjournals.org

for 14 d after birth because beyond 14 d there is a high likelihood that the animals would develop septicemia and/or pneumonia (13). Because sepsis plays a significant role in the development of chronic lung disease in preterm animals, the presence of septicemia/pneumonia in the animals would significantly alter our ability to detect differences due to other interventions. None of the animals in our control or treatment groups were infected or had pneumonia during the study period.

Oxygenation index (OI) was calculated using the following formula:

$$\text{OI} = \text{mean airway pressure (cm/H}_2\text{O)} \times \text{FiO}_2 \times 100/\text{PaO}_2$$

Similarly, ventilation index (VI) was calculated using the formula

$$\text{VI} = \text{peak inspiratory pressure} \times \text{ventilator rate} \times \text{PaCO}_2/1,000$$

Animals were randomized before delivery to either ductal ligation (ligation) on Day 6 of life or no intervention (control). This time point was chosen because this precedes the time that humans develop altered pulmonary compliance due to a persistent PDA (5, 6, 11). Pulmonary function testing was performed using the VitalTrends plethysmograph system (VT1000; VitalTrends Technology, New York, NY) (23). Compliance and resistance measurements, obtained at 6, 12, 18, 24, 36, and 48 h and every 24 h thereafter, were of the respiratory system as a whole. For analysis, compliance was corrected for bodyweight.

The technique for the echocardiographic examination of the preterm baboon has been described previously (25). Echocardiographic studies were performed at 1 and 6 h of age and at 24-h intervals, up to 1 d before necropsy.

Tracheal aspirates were collected after instillation of 1 ml of sterile normal saline through the endotracheal tube at 24 h, 48–96 h, 5–7 d, 9–11 d, and 12–14 d as previously described (23). IL-6 and IL-8 concentrations were determined by radioimmunoassay and enzyme immunoassay, respectively (23).

Post mortem pressure–volume curves were performed by the method described by Ikegami and colleagues (26). After the acquisition of the pressure–volume curve, the right lower lobe was removed, weighed, and intrabronchially fixed with phosphate-buffered 4% paraformaldehyde at 20 cm H₂O constant pressure for 24 h. After fixation, the volume of the right lower lobe was determined by volume displacement and subsequently processed for light microscopy (23).

Immunostaining for platelet endothelial cell adhesion molecule (PECAM) CD31 (Dako Corporation, Carpinteria, CA), an endothelial cell marker, was performed on lungs from both study groups and adult baboons. A semiquantitative point-counting method in which the lung parenchymal tissue served as the volume of reference was used to determine the volume fraction (Vv) of immunoreactive sites as previously described (27).

Pulmonary histopathologic comparison between the two groups was also performed by the panel of standards method as previously described (23).

Data are presented as mean $\pm$ SD. Between-group differences were compared by analysis of variance, unpaired *t* test, or the Mann-Whitney rank sum test where appropriate. Statistical results were generated using Statview (SAS Institute, San Francisco, CA) software.

RESULTS

Study Groups and Clinical Course

A total of 27 animals were studied prospectively. Two animals, randomized to the control group, had spontaneous ductus closure before Day 6 (the day of planned ductus ligation) and were excluded from the analysis. The remaining 25 animals (control = 13, ligation = 12) all had a patent ductus on Day 6. There were no differences between the two groups in birthweight (control = 393 ± 38 , ligation = 405 ± 48 g), sex (% male: control = 54%, ligation = 58%), gestation (control = 126 ± 1 , ligation = 125 ± 2 d), or in any of the measured parameters before the time of planned ductus ligation (Day 6; *see below*).

Both groups had a similar degree of left-to-right shunt through the PDA as reflected by the similar $\dot{Q}_P/\dot{Q}_S$ ratios before ductus

ligation (Day 5; Figure 1A). The ductus in the control group stayed open throughout the 14-d experiment; the average $\dot{Q}_P/\dot{Q}_S$ ratio for the control group fluctuated between 1.7 and 2.3 from Days 7 through 13 (Figure 1A). Although the control animals developed an increase in the globular shape of the cardiothymic shadow on the chest radiograph during the second week after birth, no other differences in radiographic findings were noted (e.g., alterations in lung volume, atelectasis, or increased pulmonary vascularity).

Cardiovascular Function

Before ligation on Day 6, both groups had similar systemic blood pressures, ratios of pulmonary to systemic blood flow ($\dot{Q}_P/\dot{Q}_S$), and indices of left ventricular and right ventricular performance (left ventricular = shortening fraction [SF], rate-corrected velocity of circumferential fiber shortening [VCFc]; right ventricular = acceleration time [AT], peak velocity of ejection at the pulmonary valve [Pvel(P)]).

The average $\dot{Q}_P/\dot{Q}_S$ ratio in both groups was 2.2 at 6 h after birth and fluctuated between 1.5 and 2.0 between 24 and 120 h after birth. Immediately after ductus ligation, the $\dot{Q}_P/\dot{Q}_S$ ratio

Figure 1. (A) Pulmonary-to-systemic blood flow ($\dot{Q}_P/\dot{Q}_S$), (B) mean systemic blood pressure (BP), and (C) ratio of pulmonary-to-systemic systolic BP in ligated ($n = 12$; solid circles) and control (unligated, $n = 13$; open circles) animals. Surgical ligation was performed on Day 6 in the ligated group. Values are mean $\pm$ SD. Values between the study groups were similar before ductus ligation (Day 5) in A, B, and C. Values between the study groups were significantly different for Days 7–13 (ligated vs. control, analysis of variance, $p < 0.05$) in A, B, and C.

dropped to 1 in the ligation group and remained lower than that in the control group during the 7 d after ligation (during both the early postoperative period [Days 7–10] and the later recovery period [Days 11–13]; Figure 1A).

The ligation group had higher mean systemic blood pressures (Figure 1B) and higher systolic and diastolic pressures (data not shown) at all times after ligation. The ligation animals also had lower ratios of pulmonary-to-systemic systolic pressures than the control group (Figure 1C).

The effects of ductus ligation on SF, VCFc, acceleration time (pulmonary) (AT[P]), and Pvel(P) are shown in Figure 2. By the end of the week after ligation, the animals in the control group had significantly lower indices of left and right ventricular performance than the animals in the ligation group (Figure 2).

Figure 2. Comparison of indices of right ventricular performance for (A) acceleration time (AT) and (B) peak Doppler velocity at the pulmonary valve (Pvel[P]) and left ventricular performance for (C) shortening fraction (SF) and (D) rate-corrected velocity of circumferential shortening (VCFc) in ligated (hatched bars) and control (open bars) animals. Indices were compared during three time periods: before ligation (values on Day 5), the early postoperative period (values on Days 7–10), and the late postoperative period (values on Days 11–13). Values are mean $\pm$ SD. * $p < 0.05$, ligated versus control.

Despite the poorer cardiac performance in the control group, there were no differences between the groups in fluid administration, urine output, base deficit, or in bicarbonate or dopamine/dobutamine administration (data not shown).

Pulmonary Function

In the ligation group, there was a drop in PaO_2 and an increase in OI that paralleled the decrease in pulmonary blood flow immediately after surgery. This remained stable during the week after ligation (Figure 3A). In contrast, the OI in the control group, which initially decreased during the first week after delivery, gradually deteriorated during the second week. At the time of necropsy, the OI in the control group was similar to the ligated group despite the higher index of pulmonary blood flow in the control group.

There was no difference in VI between the two groups during the preoperative or early postoperative period (7–10 d). VI tended to be lower in the ligated animals during the last 3 d of the study ($p = 0.08$; Figure 3B). Similarly, dynamic compliance tended to be higher in the ligated animals during the last 3 d of the study ($p < 0.08$; Figure 3C).

Figure 3. Comparison of (A) oxygenation index (OI), (B) ventilation index (VI), and (C) dynamic compliance per kilogram of bodyweight (C/kg) in ligated (hatched bars) and control (open bars) animals. Indices were compared during three time periods: before ligation (values on Day 5), the early postoperative period (values on Days 7–10), and the late postoperative period (values on Days 11–13). Values are mean $\pm$ SD. OI in the ligation group tended ($p < 0.09$) to be higher than that of the control group during the early postoperative period (Days 7–10) but was similar during the later part of the study (Days 11–13). VI tended to be lower ($p = 0.08$) and dynamic compliance tended to be higher ($p < 0.08$) in the ligated animals during Days 11–13 of the study.

Figure 4. Comparison of cytokines interleukin (IL-6 (A) and IL-8 (B) concentrations in tracheal aspirates obtained on Days 3–4, Days 5–7, Days 9–11, and Days 12–13 in ligated (hatched bars) and control (open bars) animals. Values are mean $\pm$ SD. There were no significant differences between the groups.

Tracheal Aspirate IL-6 and IL-8 Concentrations

At each time point evaluated, tracheal aspirate IL-6 and IL-8 values varied widely within both study groups (Figure 4). There were no consistent differences or trends between the control and ligation groups in either IL-6 or IL-8 values measured from the tracheal aspirates (Figure 4) or the necropsy lung lavage (necropsy lavage IL-6: control = 474 ± 724 , ligation = 115 ± 29 pg/ml; IL-8: control = $1,560 \pm 2,492$, ligation = 581 ± 431 pg/ml).

Static Pressure–Volume Curves

There was no difference between the groups in the *post mortem* measurement of static lung compliance (Figure 5).

Lung Histology and Immunocytochemistry

There were no differences in the pulmonary histopathologic findings between the two groups. Both groups exhibited varying degrees of inflation. Atelectasis was usually associated with the presence of mucus plugs in a few bronchiolar lumens. In the regions with well inflated parenchyma, the saccular walls were dilated and few secondary crests/alveoli were evident. A few animals in each group had thicker saccular walls with increased interstitial mononuclear cells, and in most of these sites, an increase in alveolar macrophages and a few scattered neutrophils were seen. Bronchopneumonias and septic emboli were not identified. Airway and vascular lesions were not evident. Point-count determinations of PECAM-positive saccular/alveolar endothelial cells were not significantly different between the two study

Figure 5. Comparison of static inflation and deflation lung pressure–volume curves performed after necropsy in ligated and control animals (see METHODS). Values are mean $\pm$ SD. Open circles, control inflation; open triangles, control deflation; solid circles, ligated inflation; solid triangles, ligated deflation.

groups (Vv [PECAM-positive vs. total parenchyma]: control = 0.48 ± 0.13 , $p \leq 0.05$ vs. adult; ligated = 0.41 ± 0.04 , $p \leq 0.05$ vs. adult) but were both significantly less than those in the adult baboon (adult = 0.81 ± 0.06). As expected, the pulmonary parenchymal values in the ligation and control groups were greater than that of the thin-walled adult lung (Vv parenchyma: control = 0.25 ± 0.04 , $p < 0.05$ vs. adult; ligated = 0.28 ± 0.07 , $p = 0.06$ vs. adult; adult = 0.17 ± 0.01). These findings were substantiated by the panel of standards analysis, which revealed no significant difference between the control and ligation groups (Figure 6).

Figure 6. Box and whisker plot of panel of standards comparison of ligated ($n = 12$) and control (unligated; $n = 13$) animals. Each photograph was graded independently by three different observers using the derived panel of standards (see METHODS). Shown are median (thick bar), mean (solid squares), interquartile spread (box), and range (whiskers). There were no significant differences between the groups.

DISCUSSION

Studies performed during the presurfactant era demonstrated that a persistent PDA contributes to decreased cardiac and pulmonary function in premature human infants. The current investigation is the first attempt to prospectively assess the effects of ductus ligation on cardiopulmonary function and lung histopathology in a premature animal model of respiratory distress syndrome and PDA. None of the animals received antenatal glucocorticoids and all of the animals received postnatal surfactant therapy.

Prior short-term studies have observed a decrease in pulmonary artery pressure and an increase in systemic blood pressure immediately after ductus ligation (4, 28). The current study demonstrates that these differences persist during the week after surgery (Figure 1). We also found that ductus ligation affects the indices of right and left ventricular performance. Because the indices of right ventricular function (AT[P] and Pvel[P]) are both afterload-dependent, their improvement after ductus ligation could simply be a reflection of the decrease in pulmonary pressures rather than a true change in cardiac function. On the other hand, it is possible that the difference in performance indices may reflect a true change in right ventricular function because the drop in pulmonary pressure was observed immediately after ligation, whereas the improvement in AT(P) and Pvel(P) did not occur until several days later.

The increase in left ventricular performance indices after ductus ligation most likely represents an improvement in left ventricular function because both indices (SF and VCFc) were higher in the ligation group despite a simultaneous increase in systemic blood pressure. Although there were differences between the two groups in cardiac performance indices, there were no differences between the groups in clinical parameters such as degree of metabolic acidosis, need for inotropic support, or rate of urine output.

The changes in OI during the first 2 wk after birth mimicked the changes seen in preterm human infants during the same time span (29). The OI in the ligation group tended ($p < 0.09$) to be higher than that of the control group during the early postoperative period (Days 7–10). This was most likely due to the reduction in effective pulmonary blood flow after loss of the PDA left-to-right shunt (28). In addition, postsurgical atelectasis and edema may have played a role as well. By the time of necropsy, the OIs in the ligation and control groups were similar, despite the increased effective pulmonary blood flow in the control group (Figure 3).

In contrast to the control group, the pulmonary mechanics indices (VI and dynamic respiratory compliance) showed progressive improvement in the ligation group during the week after ligation (Figure 3). This was not related to differences in pulmonary histology between the two groups. Although a PDA increases hydraulic fluid efflux from the pulmonary capillaries in the preterm lung (1, 2), previous studies have shown that its presence does not lead to an increase in net water accumulation or alterations in pulmonary mechanics during the first 3 d after birth (1, 2, 4). The progressive deterioration in VI and dynamic respiratory compliance at the end of the second week of life in the control animals may reflect a gradual accumulation of excess lung fluid in the control animals compared with the ligation animals. Future experiments that measure lung water and pulmonary wet-to-dry ratios in this model should be able to answer this question.

In addition to examining cardiopulmonary function, we performed studies to determine if a persistent PDA adds an additional inflammatory stress on the preterm lung and contributes to the development of chronic lung disease (13). We found no evidence that a persistent PDA contributed to increased pulmo-

nary production of the proinflammatory cytokines IL-6 or IL-8. Nor did we find evidence that a persistent PDA altered static lung compliance or lung histology at 2 wk after birth. Although the presence of a persistent PDA may have effects on cardiac performance and pulmonary mechanics, we found no evidence that surgical ligation of a PDA alters the evolution of histologic BPD during this time period.

It is possible that the techniques used in this investigation were not sensitive enough to detect alterations in pulmonary structure occurring as early as 2 wk after delivery. However, using the same techniques in the same animal model, we have successfully demonstrated differences in the evolution of BPD following other treatment regimens (23, 30, 31). It also is possible that the trauma of surgical ligation itself may have obscured the effects of PDA closure on postnatal lung development. Future studies using pharmacologic closure of the ductus may be able to circumvent this problem. In addition, it is possible that larger left-to-right shunts ($\dot{Q}_p/\dot{Q}_s > 3:1$) or more prolonged exposure to the PDA may cause pulmonary parenchymal abnormalities that were not evident in this model of chronic lung disease.

Our findings are consistent with the limited amount of data available from clinical studies. Although pulmonary function in preterm infants with a symptomatic PDA may benefit from ductus ligation, it remains unclear whether ductus closure has any effect on the evolution of BPD (9, 11, 12, 21). Ductus ligation requires a thoracotomy in small, critically ill infants. It is associated with serious complications, including pneumothorax, chylothorax, infection, and vocal cord paralysis, and it may be responsible for additional brain injury (32). We suggest that a careful evaluation of both the desired and achievable goals of ductus ligation be performed before committing infants to routine or early surgical closure.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank all the personnel that support the BPD Resource Center: the animal husbandry group led by Drs. D. Carey and M. Leland, the NICU staff (H. Martin, D. Correll, W. Cox, L. Kalisky, L. Nicley, R. Degan, S. Salazar), the Wilford Hall Medical Center neonatal fellows who assist in the care of the animals, and the UTHSCSA pathology staff (V. Winter, L. Buchanan, K. Symank, Y. Valdes, and K. Mendoza) who perform necropsies and morphometric studies. Francoise Mauray performed the artwork.

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